Effects of Follistatin-like Protein 1 on Myogenic Differentiation and Mitochondrial Respiration in Canine Myoblasts

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Follistatin-like Protein 1 (FSTL1) is a glycoprotein secreted by cardiac and skeletal muscle tissues under stress conditions. An elevated level of plasma FSTL1 has been observed after a single bout of exercise suggesting its potential myokine-like role involved in the crosstalk between muscle and other organs. **PURPOSE:** To investigate autocrine effects of FSTL1 on myotube differentiation, myosin heavy chain expression and mitochondrial respiration in primary canine myoblasts. **METHODS:** All animal procedures were performed in accordance with specific protocols approved by the Institutional Animal Care and Use Committee. Primary myoblasts were isolated from canine gastrocnemius muscle. Myotube differentiation was induced by a low serum condition for 4 days. Immunostainings were performed using MF20 antibody (rod-like tail region of myosin) and DAPI (nuclei); and fusion index was determined by calculating the ratio between total number of nuclei and number of nuclei within myotubes formed. RT-PCR assays were performed using primer sets precisely designed to specifically amplify each fiber type-associated myosin heavy chain isoforms (i.e. MyHC 7, 2, 1, and 4). Oxygen consumption of intact cells was measured using a Clark-type oxygen sensor; and mitochondrial respiration was measured using Seahorse XF96 analyzer. **RESULTS:** During myotube formation, FSTL1 treatment (300 ng/ml, 4 days) significantly enhanced myogenic potential determined by fusion index (~2-fold increase). There was a significant increase in MyHC 7 expression (~1.5 fold) in myotubes treated with FSTL1 during differentiation compared to non-treated myotubes, whereas no significant differences were observed in other MyHC isoforms. Chronic FSTL1 treatments (250 ng/ml, 64 hours) significantly increased oxygen consumption in both intact myoblasts and myotubes. Acute FSTL1 treatment (up to 500 ng/ml, either a single injection or 1-hour pre-incubation) had no significant effect on mitochondrial respiration. **CONCLUSION:** Our preliminary data suggest that FSTL1 enhances differentiation potential and increases oxidative metabolism in myogenic cells suggesting that FSTL1 may be an important cellular mediator for the benefits of exercise in the context of skeletal muscle adaptation.

Supported by NIH Grant RO1 HL133248